Neuron, Vol. 15, 311–321, August, 1995, Copyright © 1995 by Cell Press

Radial and Horizontal Deployment of Clonally Related Cells in the Primate Neocortex: Relationship to Distinct Mitotic Lineages

David R. Kornack and Pasko Rakic
Section of Neurobiology
Yale University School of Medicine
New Haven, Connecticut 06510

Summary

To analyze cell lineage in the rhesus monkey neocortex, we used recombinant retroviruses to label individual progenitor cells in the ventricular zone (VZ), then determined histochemically the distribution of their progeny during and after the period of cortical neurogenesis. Distribution patterns of labeled cells in the VZ suggested the coexistence of asymmetrically and symmetrically dividing progenitor cells, indicating that both postmitotic and mitotic progeny are produced during cortical neurogenesis. In the cortex, several distinct patterns of clonal distribution were observed and interpreted as follows: clones aligned radially suggested that asymmetrically dividing progenitors generate sequential "siblings" that migrate, in tandem, along a common radial path to the cortex. In contrast, clones oriented horizontally within a single lamina suggested that symmetric divisions produce multiple, laterally displaced progenitors which, in turn, simultaneously generate "cousin" cells that migrate, in concert, to the same cortical layer. Thus, we propose that different mitotic lineages, which coexist in the monkey VZ, produce distinct radial or laminar patterns of clone deployment that foreshadow the cytological organization of the adult neocortex.

Introduction

The cellular composition of the mammalian neocortex is organized into both horizontal layers and vertical columns. In the past three decades, a general picture has emerged of how this ordered, three-dimensional neuronal structure develops from a two-dimensional sheet of proliferating cells lining the lateral cerebral ventricles. During a restricted period of neurogenesis, the entire complement of cortical neurons is generated from dividing progenitor cells in the ventricular zone. After their final division in this zone, these postmitotic cells migrate outward along radial glial fascicles and settle in the developing cortical plate in an inside-out manner, such that successively later-born cortical cells occupy progressively more superficial laminar positions (reviewed in McConnell, 1988; Rakic, 1988; Volpe, 1995). These early studies of cortical histogenesis raised questions about the role of individual progenitors in generating cortical cells and about how cell production and deployment from such precursors may be related to the formation of layers and columns in the cortex.

More recently, recombinant retroviral vectors carrying reporter genes have allowed examination of cell lineage in the developing cerebral cortex in vivo (Sanes et al., 1986; Cepko, 1988; Sanes, 1988). By this method, the reporter gene is integrated into the genome of a progenitor cell infected with a replication-incompetent retrovirus and is passed without dilution to the cell's progeny; thus labeled, the lineally related progeny, i.e., the clone, can then be identified histochemically. So far, neocortical cell lineage tracing experiments have been done only in the lissencephalic rodent cerebrum and have primarily focused on issues of phenotypic commitment of the clonal progeny of precursor cells and the relationships of clonal boundaries to cortical cytoarchitectonic units (Luskin et al., 1988, 1993; Price and Thurlow, 1988; Walsh and Cepko, 1988, 1992, 1993; Grove et al., 1993; Mione et al., 1994).

Although the sequence of corticogenetic events outlined above is similar for all mammals, the neocortex varies profoundly in size and configuration among different species (Hofman, 1985; Haug, 1987; Welker, 1990), suggesting the existence of species differences in developmental mechanisms. We therefore chose to employ retroviral vectors to examine cortical cell lineage in the developing gyrencephalic cerebrum of the primate. Unlike the rodent, in which cortical neurogenesis proceeds almost until the time of birth, all cortical neurons of the rhesus monkey are produced during the middle of its 165 day gestation, between embryonic day (E) 40 and E100 (Rakic, 1974, 1976). Because the rhesus monkey cortex is approximately 100 times larger in surface area and takes about 10 times longer to develop than that of the mouse (Rakic, 1975), it potentially provides an increased spatiotemporal resolution of developmental events. Furthermore, based on electron microscopic, immunocytochemical, autoradiographic, and electrophysiological observations, the laminar and columnar domains of the neocortex appear to be more prominent and precise in primates than in rodents (Rakic, 1972, 1974, 1995a; Hubel and Wiesel, 1977; Levitt and Rakic, 1980; Gadisseux et al., 1990), allowing relationships between clonal distribution and cortical organization to be readily examined. Finally, because of similarities in cortical neural organization among primates, results from cell lineage studies in the monkey neocortex might be applicable to understanding corticogenesis in humans.

Replication-incompetent retroviruses containing the histochemical marker gene, Escherichia coli β-galactosidase (*lacZ*), were injected into the lateral ventricles of fetal rhesus monkeys at the middle of the period of cortical neurogenesis. Short- and long-term survival experiments were performed, allowing us to examine patterns of cell division and migration, as well as the eventual distribution of clonally related cortical cells. Among distribution patterns of retrovirally labeled cells in the cortex was a pattern not previously reported in lineage tracing experiments in the rodent. Based on the clonal distribution patterns we observed in the present study, specific hypotheses are proposed regarding how patterns of cell proliferation and migration dynamics may organize cell placement in the developing monkey neocortex. Specifically, we propose that the different distribution patterns of clonally related cells in the monkey neocortex may reflect distinct modes of cell division in the ventricular zone.

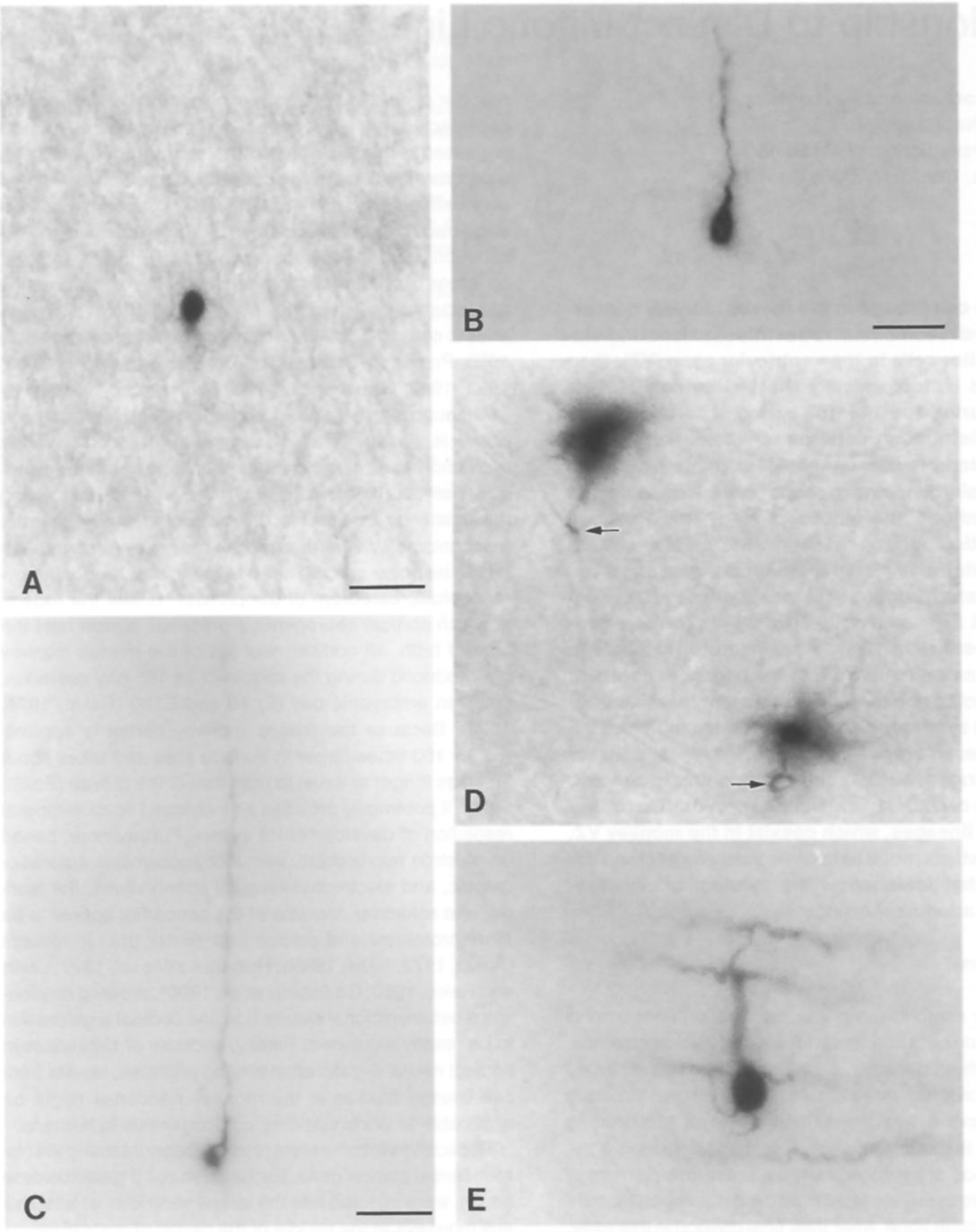

Figure 1. Classification of lacZ$^+$ Cells in the Fetal Monkey Brain Injected with Two Types of Retroviruses

(A) Example of a cortical cell with a stained nucleus (i.e., derived from an LZ12-infected progenitor).

(B–E) Examples of cells of different cell classes with stained cytoplasm (i.e., derived from a CXL87-infected progenitor).

(B and C) Cortical cells having pyramidal cell–like morphology show stain filling short (B) and long (C) apical processes.

(D) Two cells classified as protoplasmic astrocytes on the basis of their fine, bushy processes and "end feet" (arrows) contacting a blood vessel.

(E) An example of a putative myelinating white matter oligodendrocyte. The stain fills what appear to be several cytoplasmic loops of myelin that extend out of the plane of focus.

Brains were perfused at E116 (A–C) or E117 (D and E). Bars, 20 μm (A and E), 30 μm (B), 40 μm (C and D).

Results

Four fetuses (eight cerebral hemispheres) received retroviral injections between E63 and E68, when 40% of the period of cortical neurogenesis has elapsed and before the neurons of cortical layers II–IV and some of layer V have been generated (Rakic, 1975). To determine possible modes of cell division by which cortical cells are generated from individual precursors in the ventricular zone, the brain of one fetus was harvested at E89, 3 weeks after retrovirus

injections. At this age, cortical cells are still being produced, and considerable migration can be observed in the intermediate zone of the cerebral wall (Rakic, 1975). To determine the eventual distribution of retrovirally labeled cortical cells, the brains of two other fetuses were harvested approximately 7 weeks later at E117 and E116. These ages represent a stage when proliferation and migration of cortical neurons have ceased (approximately E100 and E110, respectively; Rakic 1974, 1975), but before ensuing cortical growth and expansion could potentially disrupt initial spatial relationships among postmigratory cells. The fourth fetus died in utero by 21 days after retrovirus injection.

Cell Staining and Classification

Before undertaking an analysis of cortical cell lineage, it was important to determine whether the two retroviruses used could infect and promote *lacZ* expression in cells of the monkey cerebrum, since neither virus had been used previously for studies using primate neural tissue. Previous experiments using avian precursor cells demonstrated that these two retroviruses are histochemically distinct, targeting lacZ to either the cell nucleus or the cytoplasm (see Experimental Procedures). In the present study, retrovirally labeled cells had blue histochemical reaction product confined mostly to either the cell nucleus or the cytoplasm, indicating that both types of virus had infected monkey neural cell progenitors (Figure 1). In cytoplasmic lacZ$^+$ cells, the stain often extended into cell processes, allowing phenotypic identification of a cell on the basis of its morphology. lacZ$^+$ neurons, astrocytes, and oligodendrocytes were identified, suggesting that retroviral infection and *lacZ* expression were not limited to particular cortical cell phenotypes and were compatible with their normal development (Figure 1). All lacZ$^+$ cells identified as postmigratory cortical neurons had a pyramidal cell–like appearance, with an apical process extending from the soma. This is consistent with the observation that many immature nonpyramidal as well as pyramidal neurons have such an apical process at this stage of neocortical development in the monkey (Schwartz and Meinecke, 1992). Thus, the two retroviruses were adequate for lineage analysis in the developing monkey cerebrum.

Distribution of Labeled Cells in Proliferative Zones during Cortical Neurogenesis

When cerebral hemispheres were harvested at E89, while cortical neurogenesis and neuronal migration were still in progress, two distinct distribution patterns of lacZ$^+$ cells were observed in the ventricular zone and are described below.

Solitary Cells

Single, isolated lacZ$^+$ cells observed in the ventricular zone indicated that the progeny of some dividing cells did not leave this zone during the 3 weeks following injection (Figure 2A). Since these cells had not departed for the cortex and remained in the ventricular zone, where virtually all cells are actively mitotic (Takahashi et al., 1993), they were considered to be progenitors. Occasionally, a cohort of radially deployed, spindle-shaped lacZ$^+$ cells was directly supra-adjacent to a single cell at the ventricular surface (Figures 2B and 2C), suggesting that at least some of these solitary cells were the product of an asymmetric division that remained in the ventricular zone as a "stem cell." In the intermediate zone, cytoplasmic lacZ$^+$ cells displayed the bipolar morphology typical of a migrating neuron. The "leading process" of these cells was usually tortuous rather than straightened out; this was true for cells in white as well as gray matter.

Clusters

Clusters of four or more closely apposed lacZ$^+$ cells were occasionally observed at the surface of the ventricle (Figures 2D–2F). All cells within a given cluster expressed either cytoplasmic or nuclear label, suggesting that they were the clonal progeny of a single infected progenitor. The number of lacZ$^+$ cells within a cluster suggested that they were the product of three or more rounds of symmetric cell division that occurred within the germinal zone during the interval between injection and sacrifice.

Patterns of Labeled Cells in the Neocortex after Completion of Neurogenesis

When animals were sacrificed approximately 7 weeks following retroviral injections, after cortical cell proliferation and migration have ceased, the majority of lacZ$^+$ cells were located throughout the cerebral cortex, including frontal, parietal, temporal, and occipital lobes. Labeled cells were distributed either singly or in clusters of 2 or more. Clusters were composed entirely of either nuclear- or cytoplasmic-labeled cells and were categorized according to the cell distribution patterns described below.

Radial Arrays

Each hemisphere contained arrays of 2–6 labeled cortical cells aligned radially (Figures 3A and 3B; Table 1); 54% of the total number of radial arrays were composed of only 2 cells. Because of the possibility that an array of only 2 cells might not reliably indicate a linear pattern of clone distribution, we confined further analysis to arrays of 3 or more cells (Table 1). Arrays of 3 or more cells had an average size of 3.6 cells. Most arrays spanned less than one-half of the cortical thickness and rarely extended from layer V to the border of layers II and I. Although rigid radial alignments were the most striking (Figure 3A), arrays were also observed in which cell alignment deviated slightly from the vertical axis (Figure 3B).

Horizontal Rows

In addition to radial arrays, each hemisphere contained groups of lacZ$^+$ cells that were horizontally aligned in a row and confined to a restricted stratum within a particular lamina (Figures 3C and 3D; Table 1). Horizontal arrays contained 2–10 labeled cells, with 2-cell rows comprising 56% of the total. For the reasons stated above, 2-cell rows were not considered for further analysis or interpretation. Rows of 3 or more cells had an average size of 3.9 cells, and often members of an array were separated by similar distances. (We refer to this pattern of cell distribution as a "row," acknowledging the possibility that group membership may have extended to adjacent sections, in which case, a more "three-dimensional" term would be more ap-

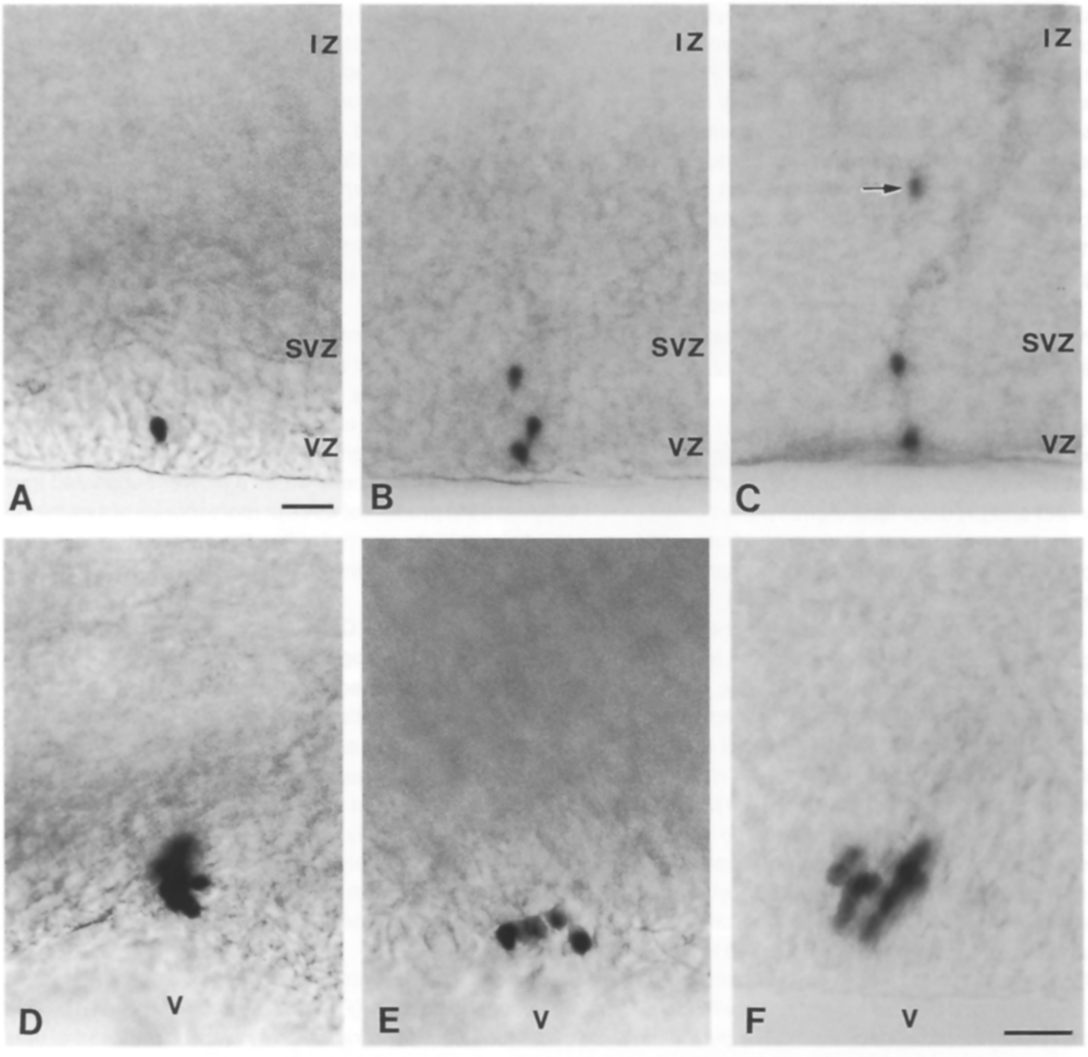

Figure 2. Distribution Patterns of lacZ$^+$ Cells in the Inner Cerebral Wall at E89, during Cortical Neurogenesis and Cell Migration

(A–C) This series of photomicrographs depicts patterns of labeled cells that are consistent with sequential stages of sibling production via asymmetric division and radial movement out of the germinal zone (see text).

(A) The location of a single labeled cell in the ventricular zone (VZ) 3 weeks after retroviral injection suggests that this is a stem cell that remained in the zone during the postinjection period.

(B) Labeling revealed 3 radially aligned cells. As in (A), the innermost cell at the ventricular surface is probably a progenitor cell; the outer 2 may be either related germinal zone progenitors or migrating cells leaving the VZ.

(C) Shown are 3 lacZ$^+$ cells in a radial line extending outward from the ventricular surface. The outermost cell (arrow), in the intermediate zone (IZ), is probably migrating; the innermost cell, a progenitor; the middle cell, at the VZ/subventricular (SVZ) border, may be either mitotic or migrating.

(D–F) Examples of clusters of closely apposed lacZ$^+$ cells in the VZ, each being the likely product of a symmetrically dividing progenitor (see text).

(D) A cluster of at least 10 labeled cells. Some of these cells lie out of the plane of focus of the photomicrograph.

(E) A 4 cell cluster showing slight tangential spread between members.

(F) A multicell cluster of at least 10 cells in the VZ. V, lateral ventricle.

Bars, 20 μm (A–E), 20 μm (F).

propriate.) When discernible by cytoplasmic label, cells within a given row had a similar morphology, e.g., displayed apical processes characteristic of young cortical neurons. Rows occupied layer II, III, or IV and did not appear to predominate in any one particular lamina. Rows were not observed in laminae I, V, or VI; since viruses were injected after the period during which the cells of these layers were generated (Rakic, 1974, 1975), it is not known whether rows are a component of these earlier generated layers.

Isolated Cells

Solitary cells expressing either nuclear or cytoplasmic label were observed between cortical layers I and V of each hemisphere examined. A cell was considered to belong to this category if it was >300 μm from another cell with the same label type (Luskin et al., 1988; Price and Thurlow, 1988). The potential significance of single retrovirally labeled cortical cells has been discussed at length in recent retroviral studies in the rodent cerebral cortex, where such cells are also found (Austin and Cepko, 1990; Mione et

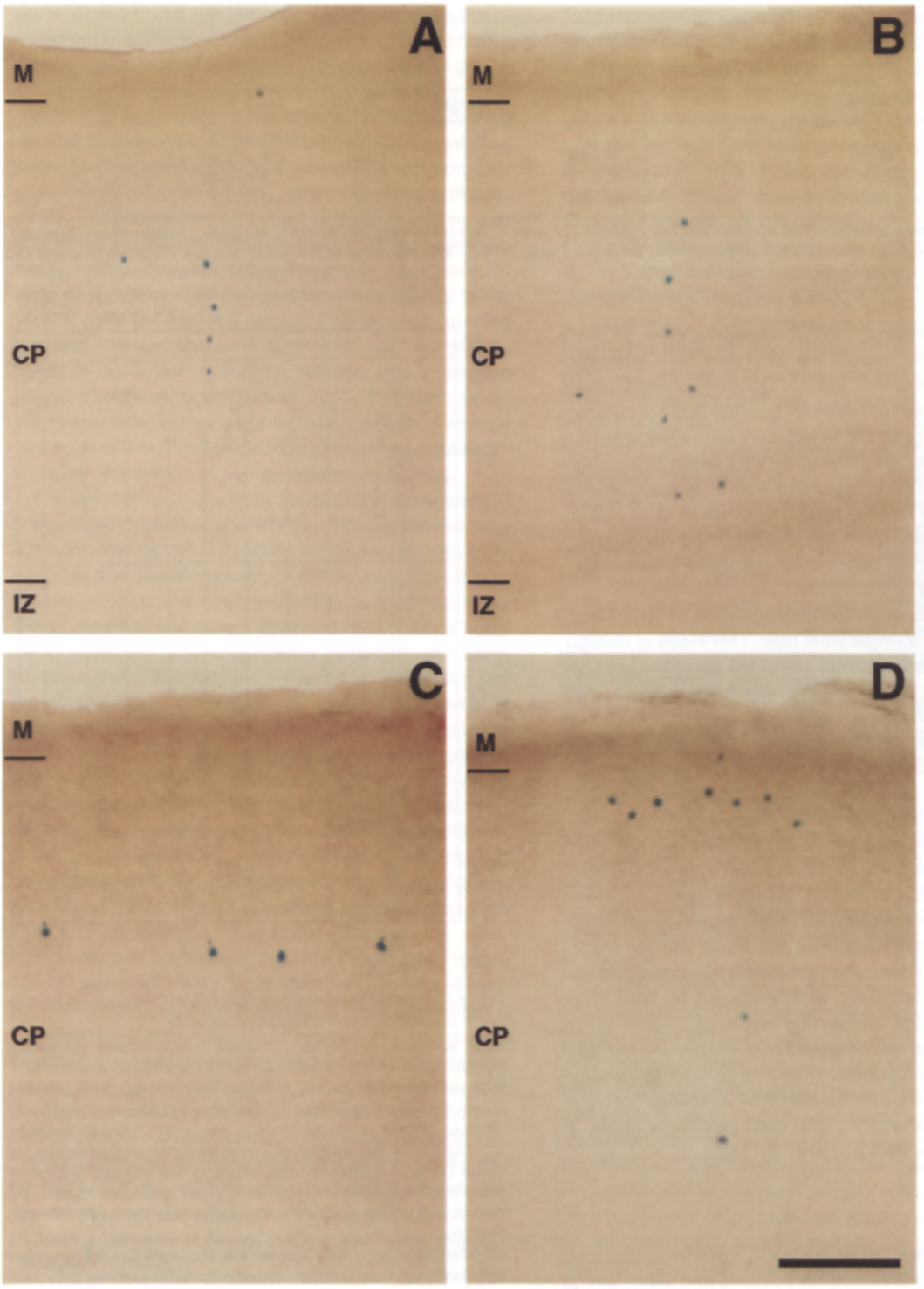

Figure 3. Distribution Patterns of lacZ$^+$ Cells in the Monkey Neocortex at E117, after Neurogenesis and Migration

(A and B) Examples of radial arrays. Degree of alignment varied from rigid, as in the 4 cell array illustrated in (A), to groupings in which some cells showed minor horizontal deviation (B).

(C and D) Examples of horizontal arrays in which labeled cells were situated in a row within a particular lamina. The bottom of each photomicrograph demarcates the middle of cortical layer VI. (C) shows an isolated 4 cell row in layer IV; (D) shows a 7 cell row in layer II.

Note that radial and horizontal alignments of lacZ$^+$ cells were reliably and repeatedly observed, suggesting that they represent biological patterns; in contrast, any occasional nearby cells that deviated from the main linear pattern, when present at all, did not show any consistent distribution pattern relative to the main array (see text). CP, cortical plate; IZ, intermediate zone; M, marginal zone (layer I). Bar, 200 μm.

Table 1. Arrays of More Than 2 lacZ+ Cells in the Neocortex after Neurogenesis and Cell Migration

Age at Injection–Age at Perfusion	Cerebral Hemisphere	Radial Arrays			Horizontal Arrays		
		n	Mean ± SD	Range	n	Mean ± SD	Range
E64–E117	Left	7	3.7 ± 1.1	3–6	17	4.2 ± 1.7	3–10
	Right	5	3.4 ± 0.55	3–4	10	4.2 ± 1.9	3–8
E68–E116	Left	2	3.5 ± 0.71	3–4	4	3.3 ± 0.50	3–4
	Right	3	3.7 ± 0.58	3–4	3	4.0 ± 1.0	3–5

Quantitative analysis was restricted to arrays of 3 or more cells; 2-cell groups were considered insufficient to establish a reliable linear alignment. The number of arrays (n), the average number of cells per group (mean ± SD), and the range of the group size are indicated for radial and horizontal arrays.

al., 1994). In the present study, a single labeled cell might be part of a clone in which the other members either contain, but do not express, the *lacZ* gene (Walsh and Cepko, 1993), are located further away than the 300 μm criterion, or may occupy positions in adjacent tissue sections. It is also possible a single cell may be the 1 postmitotic daughter that inherited the viral genome from an infected progenitor (Austin and Cepko, 1990; Hajihosseini et al., 1993). The experimental design and methods used in the present study could not discriminate among these possibilities.

Large Clusters

Occasionally, we observed larger clusters of 10–25 lacZ+ cells displaying a single label type. This mode of distribution was less common than any of the other five, and cell number among these clusters was more variable, suggesting that such groups might be composed of more than one clone. Although the distribution of labeled cells in some clusters suggested a combination of radial and horizontal patterns, most large clusters did not show an obvious orientation along columnar or laminar dimensions.

The phenotype of cells in these clusters was not determined because either only nuclei were retrovirally labeled, or the cells appeared relatively undifferentiated. However, in terms of their large size (i.e., cell number) and configuration, these clusters resembled retrovirally labeled clones of glial cells that have been observed in the rodent cerebral cortex (Luskin et al., 1988, 1993; Price and Thurlow, 1988; Grove et al., 1993), suggesting that large clusters in the monkey may also be composed of glial cells. Indeed, glial precursor cells are present in the rhesus monkey ventricular zone at the time of the retroviral injections (Levitt et al., 1983), and after E90, glial cells proliferate locally in the cortical plate (Rakic, 1974, 1975).

Discussion

Several distribution patterns of lacZ+ cells were observed in monkey cerebral hemispheres harvested either during or after the period of cortical neurogenesis. During neurogenesis, the presence of two distinct patterns of labeled cells in the ventricular zone suggested the coexistence of two different modes of cell division. After the period of neurogenesis and cell migration, cells were aligned in several different patterns in the neocortex. Of these cortical patterns, the alignment of lacZ+ cells in radial or horizontal arrays suggested specific and distinct scenarios implicating mitotic and migratory events underlying their formation.

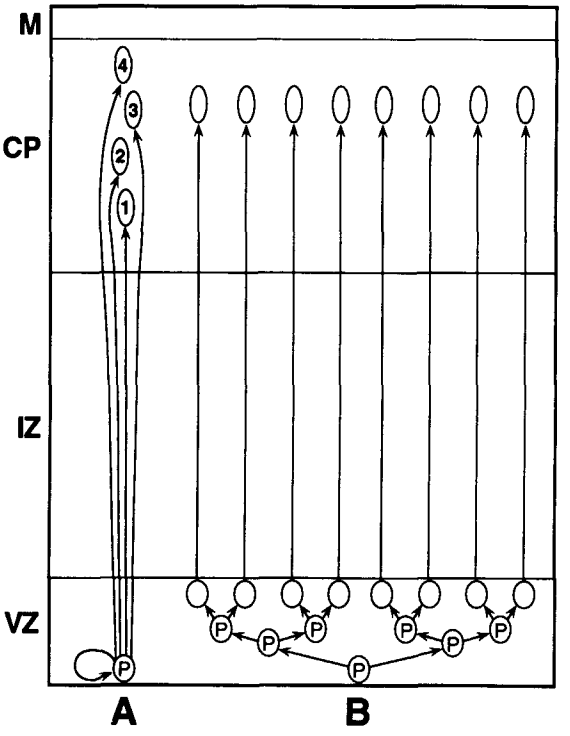

Figure 4. A Model Relating Clonal Distribution Patterns in the Neocortex to Specific Mitotic and Migratory Histories

(A) A radial array is composed of sibling cells generated sequentially from an asymmetrically dividing stem cell in the ventricular zone (VZ). With each round of mitosis, the stem cell produces 2 daughters: 1 leaves the VZ and the other remains as a self-renewing progenitor. Because of their sequential mode of production, the cells leave the VZ in tandem to occupy successively more superficial positions in the cortex (1–4). The radial ordering suggests that a coupling between the point source in the VZ and a restrictive migratory route to the cortical plate (CP) is established and maintained during cell production.

(B) A horizontal array may be composed of cousins that are generated simultaneously from multiple, related progenitors and migrate in concert to a common cortical lamina. The progenitors may be descendents of a single symmetrically dividing ancestor. The unlabeled ovals in the VZ could represent either the daughters of terminal symmetric divisions before they migrate or asymmetrically dividing cells, each generating a single row member; our results cannot discriminate between these possibilities. IZ, intermediate zone; M, marginal zone; P, proliferative cell.

Symmetric versus Asymmetric Cell Division in the Ventricular Zone

Theoretically, cells of the cerebral cortex could be generated by a mitotic lineage of either symmetric or asymmetric divisions, or some combination of both. Before the onset of cortical neurogenesis, around E40 in the macaque monkey, all progenitors in the ventricular zone divide symmetrically and, therefore, produce only other progenitors (Rakic, 1988). Evidence from [^{3}H]thymidine "birthdating" studies suggests that after E40 some progenitors divide asymmetrically, with 1 daughter cell remaining in the ventricular zone as a mitotic stem cell and the other moving to the cortical plate to become a neuron. Previous cell counting analyses inferred that both symmetric and asymmetric divisions occur during cortical neurogenesis in primate (Rakic, 1988) and rodent (Smart, 1985; Takahashi et al., 1994). However, independent evidence indicating whether or to what extent symmetric and asymmetric divisions continue into the period of neurogenesis was lacking.

The present finding of both solitary lacZ$^+$ cells and circumscribed clusters of labeled cells in the ventricular zone 3 weeks after retroviral injection indicated that both symmetric and asymmetric modes of cell division occur during the period of cortical neurogenesis in the monkey, when the labeled cortical arrays observed in this study were being generated. These cells were considered to be progenitors, since they had not departed for the cortex and remained in the ventricular zone, where virtually all cells are actively dividing (Takahashi et al., 1993). At least some of the solitary cells in the ventricular zone might represent self-renewing, asymmetrically dividing stem cells whose postmitotic siblings had left the germinal zone. This interpretation was supported by the occasional finding of 1 or 2 lacZ$^+$, spindle-shaped, radially oriented cells situated directly above a single cell attached at the ventricular surface (see Figures 2B and 2C), which suggests that such cells may be captured in the act of migration.

In contrast, most, if not all, cells in a cluster (see Figures 2D–2F) were most likely progenitors that descended from a symmetrically dividing precursor that had inherited a viral genome. Each cluster might represent several rounds of symmetric division (and thus the production of additional progenitors) after viral inheritance. It is also possible that some of the solitary cells discussed above could be progenitors that were produced by a symmetric division and displaced laterally from their sibling (see below). As discussed below, the coexistence of both modes of cell division during cortical neurogenesis has implications for interpreting patterns of clonal deployment in the monkey neocortex.

Radial Deployment of Cells Produced by Asymmetric Divisions

Labeled cells in the cerebral cortex were often distributed in strict radial alignment. The close grouping and same type of label in all cells within a given radial array suggested clonal relatedness. Moreover, the number of labeled cells within radial arrays of more than 2 cells showed little variance (see Table 1), offering further support that a given array represented a single clone (see Experimental Procedures). The existence of such radially aligned, clonally related cells was previously predicted by the radial unit hypothesis of cortical development (Rakic, 1988), which is based on evidence that newly generated neurons in the ventricular zone migrate along radially aligned glial fascicles and settle in their laminar positions in an inside-out manner (Rakic, 1972, 1974). Accordingly, the rigid radial organization of lacZ$^+$ cells in the cortex suggested that they were siblings generated sequentially from an asymmetrically dividing stem cell, and that they migrated in tandem along the same radial migration route to the developing cortical plate (Figure 4A).

Occasionally, lacZ$^+$ cells within a radial clone deviated slightly from the radial axis. Potential mechanisms underlying such deviation of sibling cells include lateral movement of progenitor cells within the ventricular zone during mitosis (Fishell et al., 1993), a lateral "switch" between adjacent glial fascicles while migrating (Rakic et al., 1974; Gadisseux et al., 1990; Misson et al., 1991), and pathway distortion from the formation of incipient cortical fissures. It is, however, remarkable that, despite these dispersive influences and having traversed a migratory distance of more than 5 mm, many sibling cells in the convoluted monkey cortex, nonetheless, form strict radial alignments. This suggests that asymmetrically dividing stem cells in the ventricular zone are each strongly coupled to a migratory route during radial array formation.

It should be noted that only 1 of the 2 daughter cells of the initial retrovirus-infected progenitor cell inherits the viral genome (Austin and Cepko, 1990; Hajihosseini et al., 1993); accordingly, only 50% of infected, asymmetrically dividing cells will produce another *lacZ*-carrying stem cell capable of leaving descendents. Therefore, the number of radial arrays generated from an infected progenitor may be twice that actually observed. In addition, this means that many "isolated" retrovirally labeled cortical cells may be part of radial clones that are not detectable by present technology.

Radially aligned clones provide experimental support for the existence of cortical "ontogenetic columns" (Rakic, 1988), which were predicted by the radial unit hypothesis. Ontogenetic columns may represent the developmental basis of prospective functional columns, which are considered to be a basic cortical processing unit important in perception, memory, and cognition (Szentágothai, 1978; Mountcastle, 1979; Eccles, 1984; Goldman-Rakic, 1984). It is notable that labeled cells within a radial array account for only a portion of the total cells within a cortical column; the unlabeled cells within a column are presumably derived from other progenitors. This finding is consistent with the polyclonal nature of ontogenetic columns, which was previously proposed on the basis of radiolabel dilution in cortical cells following exposure to [^{3}H]thymidine (Rakic, 1988).

Similar radially oriented clones have also been observed in cell lineage studies using retroviral markers in the rodent cerebral cortex (Luskin et al., 1988; Walsh and Cepko, 1988, 1993; Austin and Cepko, 1990). Radial arrays of labeled cells in these experiments were interpreted as siblings sharing a common migratory path. The use of chime-

ric mice (Nakatsuji et al., 1991) and X-inactivated mosaic mice (Tan and Breen, 1993) allowed retroviral labeling of progenitor cells at earlier stages, which resulted in larger cortical clones than those observed in the former studies. These larger clones were oriented radially, demonstrating that radial alignment appears to be the predominant mode of clonal distribution in the rodent cortex (see Rakic, 1995b, for further discussion). The present results suggest that in the monkey, too, clonally related cells migrate in a radial fashion and distribute radially in the cortex. Radial alignment of clonally related cells may anticipate the formation of the prominent columnar organization of the primate neocortex.

Horizontal Rows Resulting from Synchronous Migration of Cousin Cells

Our most striking and novel finding was the frequent grouping of labeled cortical cells into strict rows confined to a horizontal plane within a single lamina, aligned parallel to the pial surface. Horizontal rows were at least as commonly encountered as the radial arrays. Most rows were widely isolated from other labeled cells, and cells within a row displayed the same label type (cytoplasmic or nuclear), strongly suggesting descent from a common progenitor. Moreover, in arrays that contained more than 2 cells, the average number of labeled cells per row was similar among cortices (see Table 1), arguing against the possibility that a row represented random "clumping" of multiple clones. These observations suggest that a given horizontal row of $lacZ^+$ cells represented a clone derived from a single progenitor.

The strict horizontal alignment of $lacZ^+$ cells suggests a different, though not incompatible, developmental scenario than that inferred for radial array formation. One possibility is that, like the radial clones, labeled cells in a row are generated successively from asymmetric stem cell divisions, but distribute tangentially. Tangential movement of cells has been observed in vitro in the mouse ventricular zone (Fishell et al., 1993) and the intermediate zone of the ferret (O'Rourke et al., 1992). However, this scenario is inconsistent with the precise inside-out gradient of cortical neurogenesis in the rhesus monkey (Rakic, 1974, 1976). These previous [^{3}H]thymidine autoradiographic studies have shown that cortical cells that are "born" at successively later times eventually lie in progressively more superficial cortical laminae. Accordingly, if multiple siblings were produced in sequence from asymmetric divisions, they would reside in different vertical positions in the cortex, regardless of whether they move tangentially before settling into their final position. Thus, we conclude that a horizontal clone lying in a restricted portion of a single cortical lamina in the monkey does not represent siblings generated by successive, asymmetric divisions.

The sharp, inside-out gradient of layer formation in the primate neocortex does suggest an alternative hypothesis to explain how clonally related cells become aligned horizontally: the cells are born simultaneously and then migrate, in concert, to their laminar destination. Thus, we propose that labeled cells in a row are not "siblings," generated sequentially from asymmetric stem cell divisions, but rather, are "cousins," descended from a common, *symmetrically* dividing progenitor that inherited the viral genome (Figure 4B). According to this hypothesis, 2 cells within a multicell row could be either siblings cogenerated from a terminal symmetric division or cousins from 2 related, asymmetrically dividing cells that were produced by a "nonterminal" symmetric division. Our hypothesis of horizontal clone formation is consistent with the previous [^{3}H]thymidine birthdating studies noted above. Moreover, it takes into account symmetric divisions, known to occur during neurogenesis, as a putative mechanism in establishing a specific pattern of clonal allocation in the primate cerebral cortex.

According to this hypothesis, the horizontal spacing between symmetrically generated cortical row members could potentially develop at any one of several stages of row formation. First, before dividing, clonally related progenitor cells may disperse laterally within the plane of the ventricular zone. Evidence of progenitor cell dispersion in the ventricular zone has been reported in studies of the developing rodent cerebrum, using different experimental approaches (Fishell et al., 1993; Walsh and Cepko, 1993). Such movement may be due to intercalations of other progenitors produced from neighboring symmetric divisions (Kimmel et al., 1994); this means of tangential clonal distribution has been implicated in the developing avian optic tectum (Gray et al., 1988; Martínez et al., 1992) and spinal cord (Leber and Sanes, 1995). Second, during migration, cells may shift laterally across glial fascicles, as described above. Third, following migration, ongoing cortical growth processes (e.g., gliogenesis and increased vascularization) in the horizontal dimension may further separate cousin cells within a lamina.

The proposed scheme of horizontal clone formation has direct implications for laminar specification in the cortex. Neurons in each cortical layer have characteristic morphological, connectional, and biochemical phenotypes, many of which appear to be determined early, at the level of the germinal zone (McConnell, 1988; Parnavelas et al., 1991; Schwartz et al., 1991; Cohen-Tannoudji et al., 1994; Frantz et al., 1994; Mione et al., 1994; Auladell et al., 1995). Although mechanisms underlying this determination remain unknown, it appears that the laminar fate of a postmitotic neuron is determined by environmental cues during the mitotic cycle of its immediate predecessor (McConnell and Kaznowski, 1991). Our results raise the possibility that the timing of this terminal mitosis is shared among clonally related cortical cousin cells, and that it may thus be determined as early as their last common, symmetrically dividing ancestor. That is, the *timing* of a "switch" to postmitotic cell production may be determined early in a lineage of symmetric divisions. This would allow environmental determinants to "set" the laminar fate of cortical cells during only the final, neurogenetic cell divisions of that lineage. By this model, during the formation of deep cortical layers, putative progenitors of upper layer cells might continue dividing symmetrically, generating additional precursors until the onset of upper layer cell production; this is consistent with the isolated clusters of ventricular zone cells we observed during neurogenesis (at E89) and with evidence

that there are virtually no "quiescent" cells in the ventricular zone during neurogenesis (Takahashi et al., 1993). This model is also consistent with and extends the contention raised in rodent studies that separate precursors may coexist for cells of deep versus superficial cortical layers (Crandall and Herrup, 1990; Fishell et al., 1990; Krushel et al., 1993). It remains to be determined whether the horizontal and radial clones we observed are phenotypically and thus functionally distinct, subserving horizontal (intercolumnar) versus vertical (intracolumnar) aspects of cortical processing of neuronal information (Gilbert, 1983, 1992).

The hypotheses of horizontal row formation via symmetrically dividing precursors may have implications for interpreting data from previous retroviral studies in the rodent. These studies have shown evidence for tangential clonal distribution in the cerebral cortex. This mode of distribution has been interpreted as evidence of random, tangential migration of postmitotic siblings, all produced directly from a single point source in the ventricular zone, i.e., an asymmetrically dividing stem cell (Austin and Cepko, 1990; Misson et al., 1991; Walsh and Cepko, 1992, 1993; Tan and Breen, 1993). Our results suggest an alternative: tangential clone members may be cousins derived from multiple progenitors, themselves progeny of a symmetrically dividing precursor present during neurogenesis. Related progenitors may disperse laterally in the germinal zone, providing multiple point sources for a single cortical clone. Thus, cortical cells that comprise a tangential clone may migrate radially from the site of their respective and clonally related predecessors to the cortex without random, tangential movement.

Presently, it is unknown whether arrays of multiple horizontal cousins are a feature unique to the primate neocortex. This pattern of alignment has not been described in the previous retroviral studies of the rodent neocortex. It remains possible that such cousin cells are generated in the rodent ventricular zone but are less obvious in their cortical distribution, since cogenerated cells in the rodent cerebrum do not distribute in horizontal bands as sharply as do those in the primate, as revealed in [^{3}H]thymidine neuronal birthdating studies (Rakic, 1974, 1976).

Implications for Neocortical Development and Organization

Examination of *lacZ*$^+$ cells in the monkey neocortex indicated the presence of clonal distribution patterns that map along either the laminar or columnar axes of the cortex. We propose that these distinct patterns of clonally related cells reflect the different mitotic histories that produced them: a mitotic lineage of asymmetric divisions may produce a radial clone oriented within a cortical column; a lineage of symmetric divisions may generate a horizontal clone aligned within a cortical layer. Lineages of variously combined asymmetric and symmetric divisions might account for labeled cell clusters that have both columnar and laminar components of cell distribution.

The different patterns of clonally related cells we observed in the ventricular zone, indicating asymmetric and symmetric cell divisions, suggest that these distinct mitotic lineages are indeed present, and coexist during the period when the cells of radial and horizontal cortical arrays are being generated. Asymmetrically dividing stem cells have previously been implicated as the direct source of cortical neurons, in general, and of radially oriented cortical clones, in particular. In contrast, symmetric cell divisions have traditionally been considered as the means by which the number of precursor cells in the proliferative zone is established, before the onset of cortical neurogenesis. Our results suggest an additional role: a lineage of symmetric divisions that occur *during* neurogenesis coordinates the eventual distribution of clonally related cortical cells.

In the monkey neocortex, the radial arrays often spanned several laminae. Conversely, it appeared that horizontal arrays intersect multiple radial compartments of unlabeled cells. This raises the possibility that radial compartments may be composed of a mixture of radial and horizontal clones. Such interlacing of radial and horizontal clones may provide a developmental basis for both the columnar and laminar organization of the primate neocortex.

Experimental Procedures

Recombinant Retroviruses

A mixture of two histochemically distinct, replication-incompetent retroviral vectors carrying the reporter gene, Eschericia coli β-galactosidase (*lacZ*), were used to infect dividing cells. CXL87, a virus used previously to label the cytoplasm of infected cells in the chick embryo, was constructed from a spleen necrosis virus and packaged in the helper cell line, D17.2G (Mikawa et al., 1991). The other virus, LZ12, which is derived from a Maloney murine virus, was constructed with *lacZ* fused to a nuclear localization signal sequence from the simian virus 40 large tumor antigen and was packaged in the amphotropic helper cell line, PA317; infection of avian cells with this virus results in blue nuclear labeling (Galileo et al., 1990; Leber et al., 1990). Titer levels ranged from 1×10^6 to 5×10^6 active virions per milliter when tested on appropriate cell lines (QT6 quail fibroblasts for CXL87 and 3T3 murine fibroblasts for LZ12; Sanes et al., 1986).

Surgical Procedures

Four pregnant rhesus monkeys (Macaca mulatta) carrying fetuses at E63, E64, E67, and E68 were anesthetized and hysterotomized (Rakic, 1976; Rakic and Goldman-Rakic, 1985). The uterus was exposed through a midabdominal incision, and the head of the fetus was located and exposed through a small uterine incision. Each fetus received four intracerebral injections of retroviral suspension, one injection into each frontal and parietal region. The amount of retrovirus delivered to each hemisphere was proportional to that used in rodent studies (Price and Thurlow, 1988; Austin and Cepko, 1990), considering the larger cerebral volume of the monkey fetus. Prior to injection, ~340 μl of viral concentrate (70% LZ12 concentrate, 30% CXL87 concentrate) was mixed with 10 μl of polybrene (1 mg/ml) to ensure infection. Injections were performed using a hand-held Hamilton syringe fitted with a 30 G needle, and injection volumes varied from 15 to 25 μl for the E63 fetus (for a total of 85 μl), from 20 to 30 μl for the E64 fetus (for a total of 50 μl per hemisphere), and were 20 μl for the E67 and E68 fetuses (totaling 40 μl of viral suspension per hemisphere). Following injection, the fetal head was returned to the uterus, and all incisions were closed using surgical sutures (Rakic and Goldman-Rakic, 1985).

Histology

The healthy, anesthetized fetuses were delivered by cesarean section, and brains were fixed by transcardial perfusion with 2% paraformaldehyde plus 0.2% glutaraldehyde in phosphate buffer. Following dissection, brains were cut into several blocks that were postfixed for 1 hr in the same fixative, then immersed overnight in a series of graded solutions of sucrose in phosphate buffer at 4°C. The blocks were then

frozen and serially sectioned at 150 or 200 μm in the coronal plane using a freezing microtome. For *lacZ* histochemistry, sections were placed directly into a staining solution of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal; 1 mg/ml), 16 mM potassium ferrocyanide, 16 mM potassium ferricyanide, and 2 mM $MgCl_2$ in PBS. The free-floating sections were incubated overnight at room temperature, washed in PBS, and stored overnight in 2% paraformaldehyde, 2% glutaraldehyde in PBS at 4°C to avoid any gradual rise in background staining (Galileo et al., 1990). After brief washes in PBS, sections were mounted onto slides, dried, coverslipped with glycerin, and scanned at 40× under bright-field optics for lacZ-labeled cells.

We limited our search to groups of labeled cells that fell within single 150–200 μm thick coronal sections. Accurate serial reconstruction of groups that may have potentially spanned more than one tissue section was not possible in the primate cortex for several reasons. First, it was difficult to find secure landmarks in undifferentiated, unstained, and poorly vascularized fetal tissue. Second, most cell groupings occupied a small region relative to the size of the entire tissue section, so that even minimal adjustments during superimposition of adjacent sections dramatically altered the spatial relation between labeled cells. Third, large sections of primate fetal tissue do not retain their shape as well as sections from older animals during mounting and drying procedures, further making superimposition of sections difficult and unreliable. Thus, while relative "intra-section" cell positions within a given section were maintained, "inter-section" positional relationships among cells in adjacent sections could be severely disrupted, and therefore, accurate reconstructions could not be guaranteed.

Clonal Analysis

A group of $lacZ^+$ cells was considered to be a single clone only if it was widely separated from any other cluster and if all cells within the group had the same histochemical label type (i.e., the cells were derived from a single progenitor cell infected by either the CXL87 [cytoplasmic label] or the LZ12 [nuclear label] virus). A similar geographical criterion has been used for clonal analysis in previous retroviral lineage studies (Sanes et al., 1986; Turner and Cepko, 1987; Gray et al., 1988; Luskin et al., 1988), and the additional histochemical requirement has been applied more recently (Leber et al., 1990; Halliday and Cepko, 1992; Leber and Sanes, 1995). The number of clusters in a given hemisphere was relatively small (see Results and Table 1), and these were not preferentially located at the injection sites. Moreover, in no instance was an isolated cluster composed of both nucleus-positive and cytoplasm-positive cells. This suggested that each cluster of $lacZ^+$ cells we observed was derived from a single infected progenitor rather than from multiple viral "hits."

Acknowledgments

This work was supported by the United States Public Health Service. Pregnant monkeys were obtained from the breeding colony at Yale University School of Medicine and the New England Regional Primate Research Center, Southborough, Massachusetts. We are thankful to Dr. Joshua Sanes (Washington University School of Medicine, St. Louis, Missouri) for providing recombinant retroviruses and for advice in carrying out this study. We thank Drs. Lizabeth Romanski and Kenneth Wikler for critical comments on the manuscript.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *"advertisement"* in accordance with 18 USC Section 1734 solely to indicate this fact.

Received March 16, 1995; revised June 15, 1995.

References

Auladell, C., Martinez, A., Alcantar, S., Super, H., and Soriano, E. (1995). Migrating neurons in the developing cerebral cortex of the mouse send callosal axons. Neuroscience *64*, 1091–1103.

Austin, C. P., and Cepko, C. L. (1990). Cellular migration patterns in the developing mouse cerebral cortex. Development *110*, 713–732.

Cepko, C. (1988). Retrovirus vectors and their applications in neurobiology. Neuron *1*, 345–353.

Cohen-Tannoudji, M., Babinet, C., and Wassef, M. (1994). Early determination of a mouse somatosensory cortex marker. Nature *368*, 460–463.

Crandall, J. E., and Herrup, K. (1990). Patterns of cell lineage in the cerebral cortex reveal evidence for developmental boundaries. Exp. Neurol. *109*, 131–139.

Eccles, J. C. (1984). The cerebral neocortex. A theory of its operation. In Cerebral Cortex, Vol. 2, E. G. Jones and A. Peters, eds. (New York: Plenum Press), pp. 1–36.

Fishell, G., Rossant, J., and van der Kooy, D. (1990). Neuronal lineages in chimeric mouse forebrain are segregated between compartments and in the rostrocaudal and radial planes. Dev. Biol. *141*, 70–83.

Fishell, G., Mason, C. A., and Hatten, M. E. (1993). Dispersion of neural progenitors within the germinal zones of the forebrain. Nature *362*, 636–638.

Frantz, G. D., Weimann, J. M., Levin, M. E., and McConnell, S. K. (1994). Otx1 and Otx2 define layers and regions in developing cerebral cortex and cerebellum. J. Neurosci. *14*, 5725–5740.

Gadisseux, J.-F., Kadhim, H. J., van den Bosch de Aguilar, P., Caviness, V. S., and Evrard, P. (1990). Neuron migration within the radial glial fiber system of the developing murine cerebrum: an electron microscopic autoradiographic analysis. Dev. Brain Res. *52*, 39–56.

Galileo, D. S., Gray, G. E., Owens, G. C., Majors, J., and Sanes, J. R. (1990). Neurons and glia arise from a common progenitor in chicken optic tectum: demonstration with two retroviruses and cell type–specific antibodies. Proc. Natl. Acad. Sci. USA *87*, 458–462.

Gilbert, C. D. (1983). Microcircuitry of the visual cortex. Annu. Rev. Neurosci. *6*, 217–247.

Gilbert, C. D. (1992). Horizontal integration and cortical dynamics. Neuron *9*, 1–13.

Goldman-Rakic, P. S. (1984). Modular organization of prefrontal cortex. Trends Neurosci. *7*, 419–424.

Gray, G. E., Glover, J. C., Majors, J., and Sanes, J. R. (1988). Radial arrangement of clonally related cells in the chicken optic tectum: lineage analysis with a recombinant retrovirus. Proc. Natl. Acad. Sci. USA *85*, 7356–7360.

Grove, E. A., Williams, B. P., Da-Qing, L., Hajihosseini, M., Friedrich, A., and Price, J. (1993). Multiple restricted lineages in the embryonic rat cerebral cortex. Development *117*, 553–561.

Hajihosseini, M., Iavachev, L., and Price, J. (1993). Evidence that retroviruses integrate into post-replication host DNA. EMBO J. *12*, 4969–4974.

Halliday, A. L., and Cepko, C. L. (1992). Generation and migration of cells in the developing striatum. Neuron *9*, 15–26.

Haug, H. (1987). Brain sizes, surfaces, and neuronal sizes of the cortex cerebri: a stereological investigation of man and his variability and a comparison with some mammals (primates, whales, marsupials, insectivores, and one elephant). Am. J. Anat. *180*, 126–142.

Hofman, M. A. (1985). Size and shape of the cerebral cortex in mammals. I. The cortical surface. Brain Behav. Evol. *27*, 28–40.

Hubel, D. H., and Wiesel, T. N. (1977). Functional architecture of macaque monkey visual cortex. Proc. R. Soc. Lond. (B) *198*, 1–59.

Kimmel, C. B., Warga, R. M., and Kane, D. A. (1994). Cell cycles and clonal strings during formation of the zebrafish central nervous system. Development *120*, 265–276.

Krushel, L. A., Johnston, J. G., Fishell, G., Tibshirani, R., and van der Kooy, D. (1993). Spatially localized neuronal cell lineages in the developing mammalian forebrain. Neuroscience *53*, 1035–1047.

Leber, S. M., and Sanes, J. R. (1995). Migratory paths of neurons and glia in the embryonic chick spinal cord. J. Neurosci. *15*, 1236–1248.

Leber, S. M., Breedlove, S. M., and Sanes, J. R. (1990). Lineage, arrangement, and death of clonally related motoneurons in chick spinal cord. J. Neurosci. *10*, 2451–2462.

Levitt, P., and Rakic, P. (1980). Immunoperoxidase localization of glial fibrillary acidic protein in radial glial cells and astrocytes of the developing rhesus monkey brain. J. Comp. Neurol. *193*, 815–840.

Levitt, P., Cooper, M. L., and Rakic, P. (1983). Early divergence and changing proportions of neuronal and glial precursor cells in the pri-

mate cerebral ventricular zone. Dev. Biol. *96*, 472–484.

Luskin, M. B., Pearlman, A. L., and Sanes, J. R. (1988). Cell lineage in the cerebral cortex of the mouse studied in vivo and in vitro with a recombinant retrovirus. Neuron *1*, 635–647.

Luskin, M. B., Parnavelas, J. G., and Barfield, J. A. (1993). Neurons, astrocytes, and oligodendrocytes of the rat cerebral cortex originate from separate progenitor cells: an ultrastructural analysis of clonally related cells. J. Neurosci. *13*, 1730–1750.

Martínez, S., Puelles, L., and Alvarado-Mallart, R. M. (1992). Tangential neuronal migration in the avian tectum: cell type identification and mapping of regional differences with quail/chick homotopic transplants. Dev. Brain Res. *66*, 153–163.

McConnell, S. K. (1988). Development and decision making in the mammalian cerebral cortex. Brain Res. Rev. *13*, 1–23.

McConnell, S. K., and Kaznowski, C. E. (1991). Cell cycle dependence of laminar determination in developing cerebral cortex. Science *254*, 282–285.

Mikawa, T., Fischman, D. A., Dougherty, J. P., and Brown, A. M. C. (1991). *In vivo* analysis of a new *lacZ* retrovirus vector suitable for cell lineage marking in avian and other species. Exp. Cell Res. *195*, 516–523.

Mione, M. C., Danevic, C., Boardman, P., Harris, B., and Parnavelas, J. G. (1994). Lineage analysis reveals neurotransmitter (GABA or glutamate) but not calcium-binding protein homogeneity in clonally related cortical neurons. J. Neurosci. *14*, 107–123.

Misson, J. P., Austin, C. P., Takahashi, T., Cepko, C. L., and Caviness, V. S., Jr. (1991). The alignment of migrating neural cells in relation to the murine neopallial radial glial fiber system. Cereb. Cortex *1*, 221–229.

Mountcastle, V. B. (1979). An organizing principle for cerebral function: the unit module and the distributed system. In The Neurosciences: Fourth Study Program, F. O. Schmitt and F. G. Worden, eds. (Cambridge: MIT Press), pp. 21–42.

Nakatsuji, N., Kadokawa, Y., and Hirofumi, S. (1991). Radial columnar patches in the chimeric cerebral cortex visualized by use of mouse embryonic stem cells expressing β-galactosidase. Dev. Growth Diff. *33*, 571–578.

O'Rourke, N. A., Dailey, M. E., Smith, S. J., and McConnell, S. K. (1992). Diverse migratory pathways in the developing cerebral cortex. Science *258*, 299–302.

Parnavelas, J. G., Barfield, J. A., Franke, E., and Luskin, M. B. (1991). Separate progenitor cells give rise to pyramidal and nonpyramidal neurons in the rat telencephalon. Cereb. Cortex *1*, 463–468.

Price, J., and Thurlow, L. (1988). Cell lineage in the rat cerebral cortex: a study using retroviral-mediated gene transfer. Development *104*, 473–482.

Rakic, P. (1972). Mode of cell migration to the superficial layers of fetal monkey neocortex. J. Comp. Neurol. *145*, 61–84.

Rakic, P. (1974). Neurons in rhesus monkey visual cortex: systematic relation between time of origin and eventual disposition. Science *183*, 425–427.

Rakic, P. (1975). Timing of major ontogenetic events in the visual cortex of the rhesus monkey. In Brain Mechanisms in Mental Retardation, N. A. Buckwald and M. Brazier, eds. (New York: Academic Press), pp. 3–40.

Rakic, P. (1976). Differences in the time of origin and in eventual distribution of neurons in areas 17 and 18 of visual cortex in rhesus monkey. Exp. Brain Res *1* (Suppl.), 244–248.

Rakic, P. (1988). Specification of cerebral cortical areas. Science *241*, 170–176.

Rakic, P. (1995a). Corticogenesis in human and nonhuman primates. In The Cognitive Neurosciences, M. S. Gazzaniga, ed. (Cambridge: MIT Press), pp. 127–145.

Rakic, P. (1995b). Radial versus tangential distribution of clonally related cells in the cerebral cortex. Proc. Natl. Acad. Sci. USA, in press.

Rakic, P., and Goldman-Rakic, P. S. (1985). Use of fetal neurosurgery for experimental studies of structural and functional brain development in non-human primates. In Prenatal Neurology and Neurosurgery, R. T. Thompson and J. R. Green, eds. (Hampton, Virginia: Spectrum Press), pp. 1–15.

Rakic, P., Stensas, L. J., Sayre, E. P., and Sidman, R. L. (1974). Computer-aided three-dimensional reconstruction and quantitative analysis of cells from serial electron microscopic montages of foetal monkey brain. Nature *250*, 31–34.

Sanes, J. R. (1988). Analysing cell lineage with a recombinant retrovirus. Trends Neurosci. *12*, 21–28.

Sanes, J. R., Rubinstein, J. L. R., and Nicolas, J.-F. (1986). Use of a recombinant retrovirus to study post-implantation cell lineage in mouse embryos. EMBO J. *5*, 3133–3142.

Schwartz, M. L., and Meinecke, D. L. (1992). Early expression of GABA-containing neurons in the prefrontal and visual cortices of rhesus monkeys. Cereb. Cortex *2*, 16–37.

Schwartz, M. L., Rakic, P., and Goldman-Rakic, P. S. (1991). Early phenotype expression of cortical neurons: evidence that a subclass of migrating neurons have callosal axons. Proc. Natl. Acad. Sci. USA *88*, 1354–1358.

Smart, I. H. M. (1985). A localised growth zone in the wall of the developing mouse telencephalon. J. Anat. *140*, 397–402.

Szentágothai, J. (1978). The neuron network of the cerebral cortex: a functional interpretation. Proc. R. Soc. Lond. (B) *201*, 219–248.

Takahashi, T., Nowakowski, R. S., and Caviness, V. S., Jr. (1993). Cell cycle parameters and patterns of nuclear movement in the neocortical proliferative zone of the fetal mouse. J. Neurosci. *13*, 820–833.

Takahashi, T., Nowakowski, R. S., and Caviness, V. S., Jr. (1994). Mode of cell proliferation in the developing mouse neocortex. Proc. Natl. Acad. Sci. USA *91*, 375–379.

Tan, S.-S., and Breen, S. (1993). Radial mosaicism and tangential cell dispersion both contribute to mouse neocortical development. Nature *362*, 638–640.

Turner, D. L., and Cepko, C. L. (1987). A common progenitor for neurons and glia persists in rat retina late in development. Nature *328*, 131–136.

Volpe, J. J. (1995). Neuronal proliferation, migration, organization, and myelination. In Neurology of the Newborn, 3rd edition, J. J. Volpe, ed. (Philadelphia, Pennsylvania: W. B. Saunders Company), pp. 43–92.

Walsh, C., and Cepko, C. L. (1988). Clonally related cortical cells show several migration patterns. Science *241*, 1342–1345.

Walsh, C., and Cepko, C. L. (1992). Widespread dispersion of neuronal clones across functional regions of the cerebral cortex. Science *255*, 434–440.

Walsh, C., and Cepko, C. L. (1993). Clonal dispersion in proliferative layers of developing cerebral cortex. Nature *362*, 632–635.

Welker, W. (1990). Why does cerebral cortex fissure and fold? A review of determinants of gyri and sulci. In Cerebral Cortex, Vol. 8B, E. G. Jones and A. Peters, eds. (New York: Plenum Press), pp. 3–136.